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STUDIES ON THE HYPERTROPHIC DISEASES CAUSED BY TAPHRINA SPECIES
II. A PLANT GROWTH SUBSTANCE EXCRETED BY THE PATHOGENS.

By

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Introduction

Generally, it has been considered that the hyperplasia of the plant is caused by stimulation of hormone produced by the parasite. Broun and Laskaris (1942) reported that indole-3-acetic acid is related to the tumour formation. And many researchers have supported their opinion. Since the IAA level rose in the tumour tissue, the cause of tumour formation was attributed to the hyperauxinity in the affected host tissue.

On the hyperplasia caused by *Taphrina* species, Hattori and Kinoshita (4) reported that the fungal bodies of *Taphrina cerasi* contained a substance which inhibited intensively the root elongation of Avena.

Thereafter, Hirata (7) reported that the *Taphrina* sp. excreted IAA in the Czapek-Dox's nutrient solution which was added *l*-tryptophan instead of KNO_3 as a nitrogen source. And we also recognized this phenomenon.

But, in the hypertrophic diseases caused by *Taphrina* sp., there are phenomena which are unable to be explained only by IAA excess. In the witches' bloom disease the twigs were abnormally generated. On the other hand, it was clarified that IAA has a character causing "apical dominance" as noted in many reports. And if so, it is reasonable to imagine that IAA would inhibit the occurrence of witches' bloom rather than stimulate.

Furthermore, *T. deformans*, *T. pruni* and *T. mume* cause abnormal division and enlargement of the cell (8). But, IAA does not promote directly the cell division.

Therefore, we imagined that there will be some other causal agents different from IAA.

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In 1961 the authors detected an active substance from the culture solution of *Taphrina* species (*Taphrina deformans* Tul., *T. pruni* Tul., *T. cerasi* Sadeb., *T. mume* Nishida) and extracted, purified and crystallized it*. The results of these experiments will be presented in this paper.

Experiments

I. The excretion and purification of the causal agent

a. Material and Method

Although each of four fungi of *Taphrina* sp. excreted the substance as stated above, *Taphrina cerasi* was used in this experiment. Because this fungus grows most vigorously in the nutrient solution among the four fungi (9, 14).

The fungus was cultured in the following culture solution

Na-glutamic acid	5.0g
KH_2PO_4	1.0g
$MgSO_4$	0.5g
soluble starch	50.0g
$FeCl_3$	trace
deionized water	1,000*ml*

The culture was aerated in darkness for 10 days at 25°C by using the apparatus shown in Fig. 1.

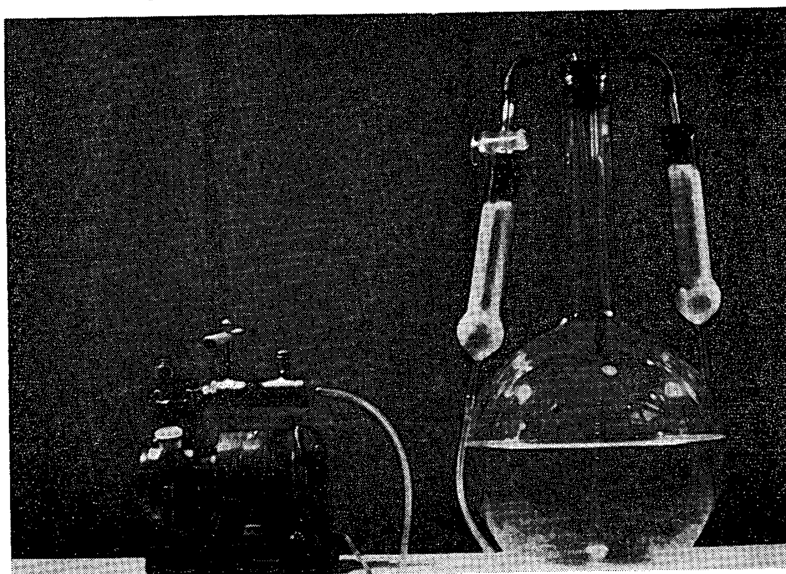

Fig. 1. The apparatus for culture.
Note : left = compressor
right = culture vessel

After the cultivation, the cultural solution was centrifuged at 3,000 r.p.m. for a few minutes and the fungal bodies were discarded. The supernatant was gathered and the substance was extracted by using the method described in Fig. 2.

* The outline of these results was already published (10) (11).

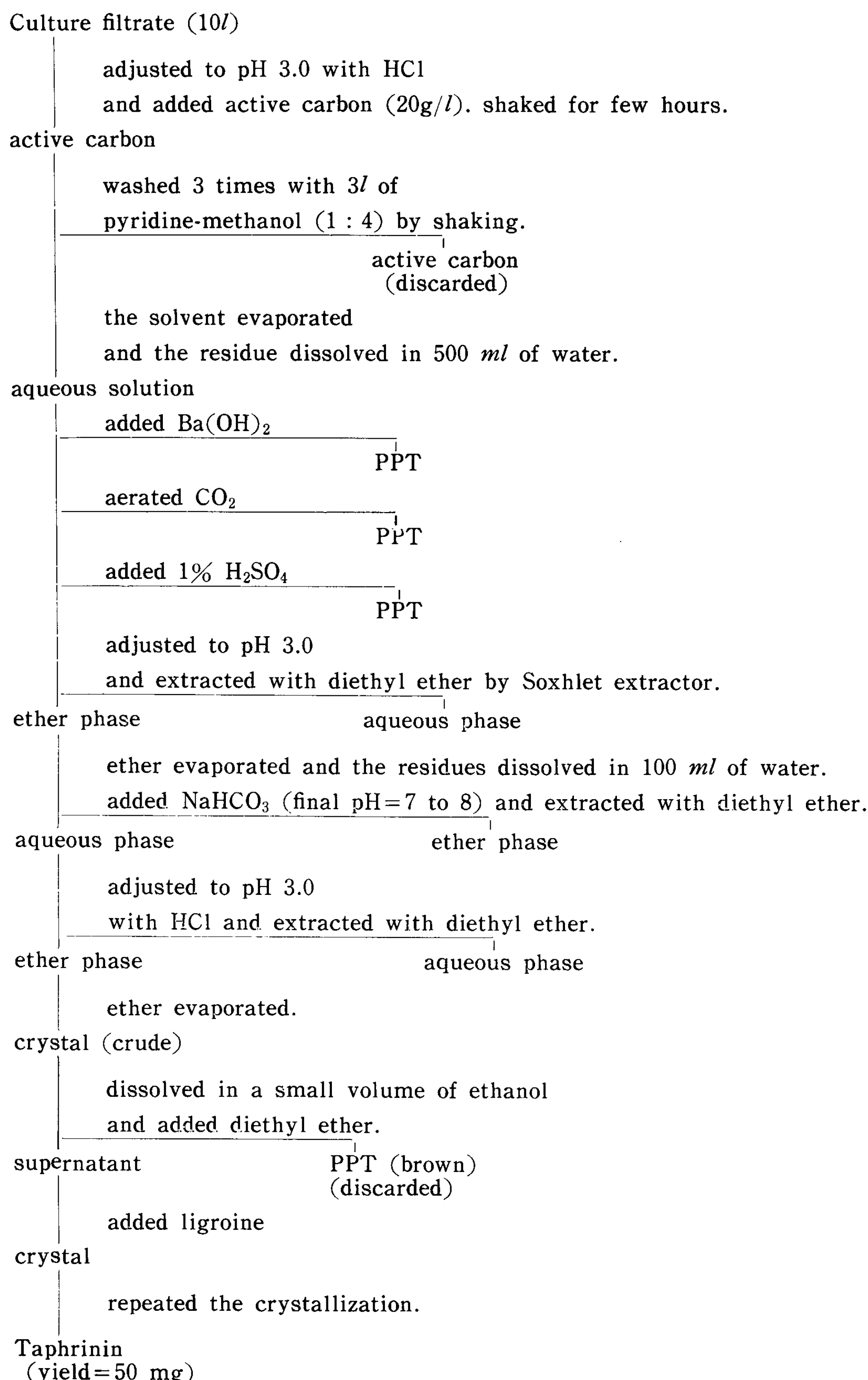

Fig. 2. The method of purification

For the purpose of testing the purity of the substance, the measurement of the melting point and the paper chromatography were employed.

b. Result

The crystal was obtained at the rate of 50 mg from 10 l of the cultural

solution. The crystal is colorless and has a short pillar shape as shown in Fig. 3. This substance will be named "Taphrinin".

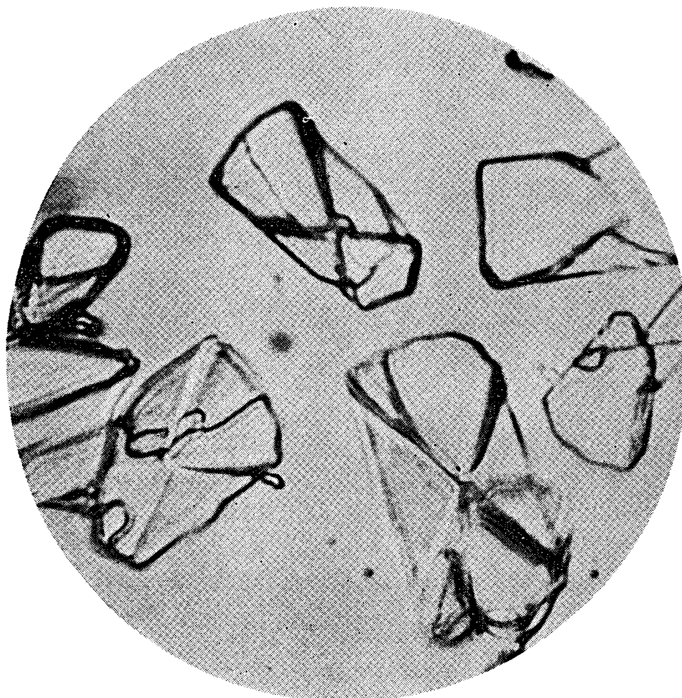

Fig. 3. The crystal of Taphrinin

II. The physical and chemical natures of Taphrinin.

1. The detection of Taphrinin on the paper chromatogram and its Rf value.

a. Method

The sample was spotted on the Toyo No. 50 filter paper (2 × 40 cm) and the paper strip was developed by the one-dimentional ascending method for 16-18 hrs at 20-22°C with the following solvents.

solvents :

a) *iso*propanol : ammonia : water = (8 : 1 : 1)··············v/v

b) *n*-butanol : acetic acid : water = (4 : 1 : 1)··············v/v

Then the strips were dried at room temperature and sprayed with the following spraying reagents.

spraying reagents: 1 per cent $FeCl_3$, 0.05 per cent ninhydrin-butanol solution, ammoniacal silver nitrate solution, 0.05 per cent BPB solution (citric acid added), Gordon-Weber's reagent, Millon's reagent, Acid-Sugar reagent(for di-, tri-phenol).

b. Results

As shown in Table 1, the *Rf* value was 0.17 at *iso*propanol-ammonia-water solution and 0.74 at butanol-acetic acid-water solution.

The spot of the substance was visible with 1 per cent $FeCl_3$, 0.05 per cent ninhydrin, ammoniacal silver nitrate solution and 0.05 per cent BPB (citric acid

Table 1. The color reaction and *Rf* value of Taphrinin on the paper chromatogram.

reagents \ solvents	Pr-Am-W(8 : 1 : 1)*	Bu-Ac-W(4 : 1 : 1)**
1% $FeCl_3$	light orange	light pink
0.05% Ninhydrin	light pink	light pink
Ammoniacal $AgNO_3$	white on brownish ground	white on brownish ground
0.05% BPB(+citric a.)	blue	――――
Gordon-Weber	negative	negative
Millon	negative	negative
Acid-Sugar	negative	negative
Rf value	0.17	0.74

Note : * isopropanol-ammonia-water (8 : 1 : 1)
**n-butanol-acetic acid-water (4 : 1 : 1)

added). On the other hand, it was invisible with Gordon-Weber's reagent, Millon's reagent and Acid-Sugar reagent.

2. Other natures

1). The substance is soluble in water, ethanol, methanol and acetone ; slightly soluble in ether, butanol, propanol and ethyl acetate ; insoluble in ligroine, petroleum ether, chloroform and xylene.

2). The aqueous solution of Taphrinin is acidic and the crystal dissolves in dilute sodium bicarbonate solution with bubbling.

3). Biuret reaction is negative.

4). The substance is hydrolysed by 6*N* HCl in 24 hrs at 110°C.

5). The melting point is 177-178°C and the substance is decomposed while melting.

6). The substance has a nature of sublimation.

7). The ultraviolet absorption spectrum was examined, and the aqueous solution of Taphrinin did not exhibit any absorption maximum between the wave length of 230 mμ and 310 mμ.

III. The effects of Taphrinin on the plants.

Effects of Taphrinin upon the organ or tissue of the plants will be published later, so in this report the outline of the effects are reported.

1. The stimulative effect on the elongation and development of axillary bud of bean.

a. Materials and Method

Bean (*Phaseolus vulgaris.* Varieties : Kinugasa and Kentucky wonder) was used in this experiment.

Soon after the development of the primary leaves, a small cotton ball was put on the leaf axil. And then a few drops of the aqueous solutions of Taphrinin of various concentrations such as 5, 10, 20, 100 p.p.m., were dropped on the cotton balls. Control plants were treated with water alone. The treatment was done

twice a day for a week.

b. Result

At 15 or 20 days after the treatment, the axillary buds began to elongate and developed. And also the flower buds were set on the newly formed shoots (Figs. 5, 7, 8, 9.). Those two varieties were affected similarly. And these phenomena were not observed in the check plant (Figs. 4, 6).

The optimal concentration was in the range from 10 p.p.m. to 20 p.p.m. and 5 p.p.m. was slightly effective. But in the concentration of 100 p.p.m. the development of axillary buds were inhibited.

It was occasionally observed that the shape of the trifoliar leaves became abnormal i.e., the lack of one leaflet or the adhesion of leaflets.

2. The promotion of main root elongation

a. Materials and Method

Seed of pea (*Pisum sativum.* Variety : Alaska) and flax (*Linum ustatissium.* Variety : Uylla) were germinated respectively in petri dishes at 25°C. At 48 hrs after germination, the seedlings which grew uniformally were selected. Ten selected plants as one group were transferred on the filter papers set at the bottom of the petri dish (4.5 cm in radius). Then Taphrinin solution were poured into the petri dish at the rate of 5 ml per one dish. The concentration of the solution was 5, 10, 20 and 100 p.p.m..

At 24 hrs after the treatment, the length of root was measured and the effects of the Taphrinin were investigated.

b. Results

Taphrinin promoted the root elongation of both plants as shown in Table 2 and Fig. 10.

Table 2. The effect on the elongation of flax root.

	check	5 p.p.m.	10 p.p.m.	20 p.p.m.	100 p.p.m.
1	5.5 cm	5.8 cm	8.0 cm	7.1 cm	6.2 cm
2	5.8	5.8	7.2	7.1	5.5
3	5.5	6.2	6.4	6,8	6.0
4	4.8	5.1	6.7	7.8	5.8
5	5.8	5.4	6.8	7.3	5.6
6	5.7	6.1	7.1	8.0	4.9
7	5.5	4.9	7.5	7.5	4.9
8	5.9	4.6	6.4	7.2	5.8
9	5.4	5.8	6.3	7.1	6.2
10	5.1	5.5	7.2	7.7	5.8
$X \pm S\bar{x}$	5.5±0.4	5.5±0.5	7.0±0.5	7.4±0.4	5.7±0.5

The effective concentration was in the range of 10-20 p.p.m.. But, the root elongation was rather inhibited in the concentration of 100 p.p.m.. The stimulated roots were more slender than the untreated ones.

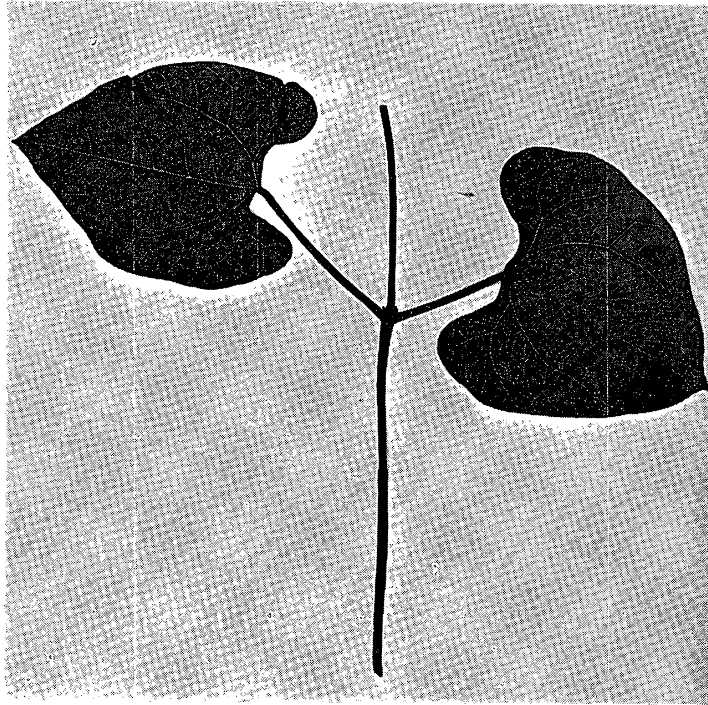

Fig. 4. check

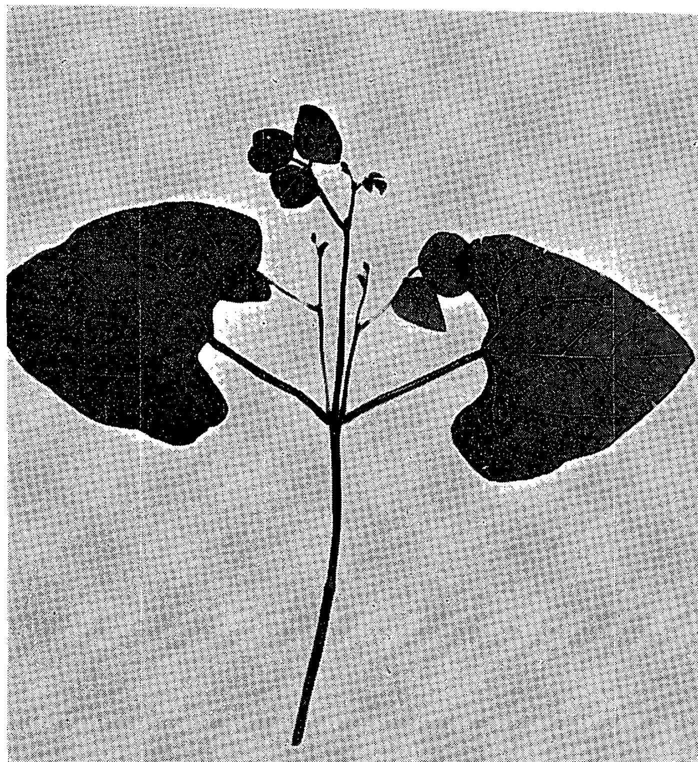

Fig. 5. treated

Figs. 4 and 5. The effect of Taphrinin on the elongation and development of axillary buds.
Variety : Kentucky wonder

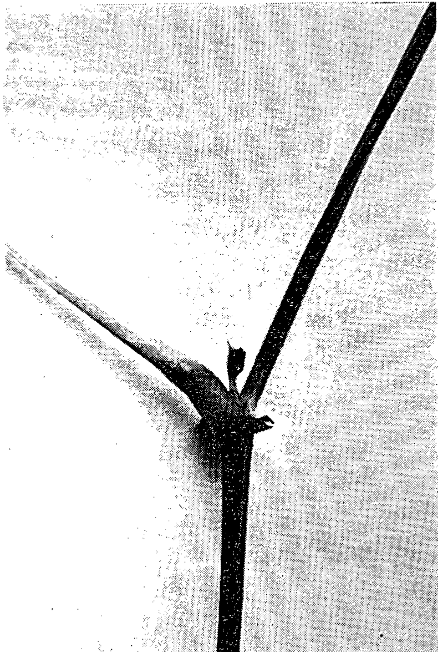

Fig. 6. check

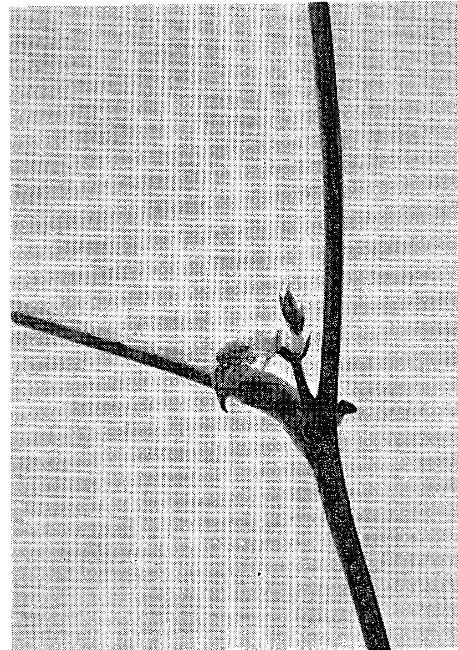

Fig. 7. 5 p.p.m.

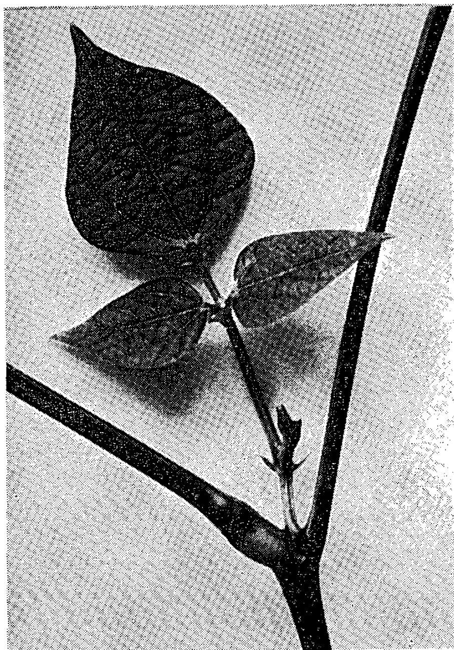

Fig. 8. 10 p.p.m.

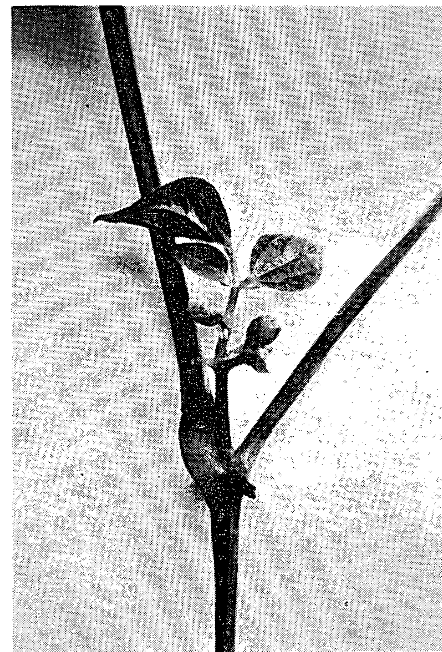

Fig. 9. 20 p.p.m.

Figs. 6-9. The effect of Taphrinin in various concentration on the elongation and development of axillary buds.
Variety : Kinugasa

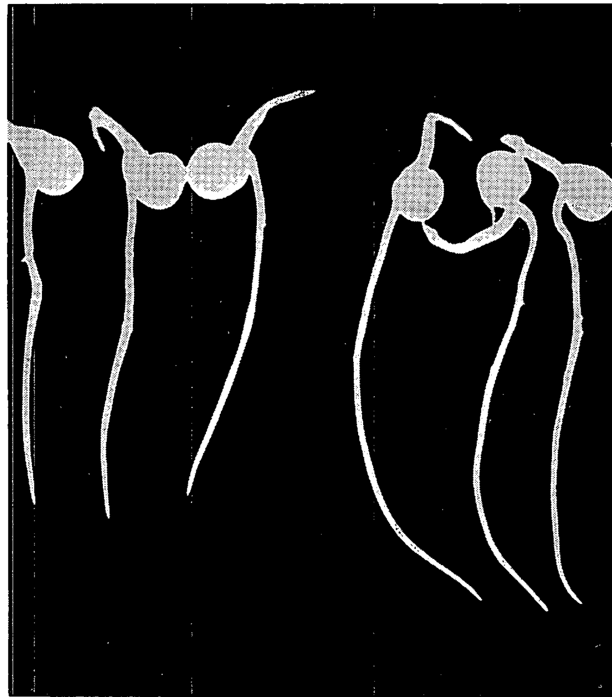

Fig. 10. The promotive effect on the elongation of pea root.
Note : left three = check
right three = treated

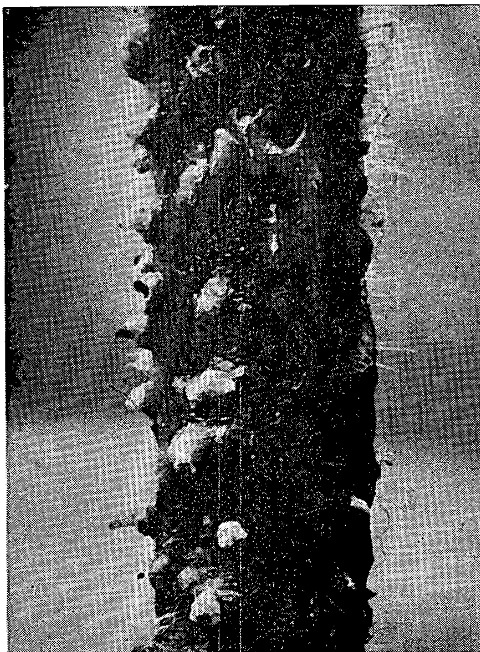

Fig. 11. check

Fig. 12. treated

Figs. 11 and 12. The effect on the aerial root genesis of tomato.
Variety : Ponderosa

3. The stimulation upon the aerial root genesis of tomato.

a. Materials and Method

Two varieties of tomato (*Lycopersicon esculentum.* Varieties: Kurihara and Ponderosa) were used. At 10 days after germination, the three seedlings were transplanted in each clay pot containing the soil.

At 20 days after transplantation, the plants were treated with lanolin paste added 5 mg of the Taprinin per 1 g of lanolin, This paste was plastered around the stem between cotyledons and the primary leaf. In the check the plants were treated with lanolin alone.

b. Result

About 20 days after the treatment, the thick and short roots were generated from the treated part of the stem (Fig. 12).

The degree of response to the Taphrinin of the varieties seemed to be slightly different from each other. The number of aerial root generated on Ponderosa was more than that of Kurihara. In the check, no root generated (Fig. 11).

4. The thickening of the leaf tissue

a. Material and Method

Bryophyllum calycinum was used for this experiment. The upper surface of the youngest leaf was plastered with the same lanolin as previously described. In the check, the leaf surface was plastered with lanolin alone.

b. Result

At 20 days after the treatment, the treated leaves became 1.5 to 2 fold thicker than the check.

Discussion and Conclusion

It has been considered that the hyperplasia of plant was caused by the excess of auxin content in affected plant tissues. And the evidence was shown by many investigators. For example, Pilet (12) reported that the auxin content of *Euphorbia* tissue which was infected by *Uromyces pisi* became 100 fold than that of the healthy tissue. And in *Sempervivum* tissue which was affected by *Endophyllum*, it became 1,000 fold.

The same phenomena were detected by Hirata(6), Daly and Inman (2), and others. Although there is Riker's experiment(13) which denies the hyperauxinity, it is an established theory that the auxin level rises in the tumour tissue.

As stated in the introduction, however, it is doubtful to attribute all causes of the hyperplasia to the hyperauxinity. At least, in the hyperplasia caused by *Taphrina* species, it cannot be explained with the IAA excess. And so, it is supposed that the hyperplasia by *Taphrina* species is caused by agents distinct from IAA.

As stated above, the Taphrinin stimulated the development of the axillary buds and the thickening of leaf tissue, and accelarated the elongation of the root.

These effects are similar to those of anti-auxin. Namely, Galston (3) reported that the 2,3,5-triiodo benzoic acid (one of the anti-auxin) stimulated the development of the axillary buds of the soybean. And Audus (1) said that diluted anti-auxin solution promoted the elongation of the root.

Since the symptoms of the diseases caused by *Taphrina* sp. are quite characteristic by each fungi, another causal agent might exist. But, *T. pruni-subcorditae* caused the plum pocket on the *Prunus subcorditae* Benth. and when infected the Indian and French prunus it causes the witches' bloom and leaf curl (5). These facts make us imagine that various symptoms caused by each fungi do not depend upon the differerences of the causal substance itself, but on the difference of sensitivity of host, on the differences of the infected portion or on the manner of parasitism.

Because of the differences in the melting point, *Rf* value and color reaction on paper chromatogram, the stimulative effects on the various plants and other natures, it was concluded that the Taphrinin is distinct from IAA. And, by the reason of the resemblance between the both symptoms caused artificially with Taphrinin and by the causal fungi, it was considered that Taphrinin is a causal principle of the symptoms caused by *Taphrina* species.

Summary

A plant growth substance was detected in the culture filtrate of *Taphrina* species(*T. deformans*, *T. pruni*, *T. cerasi*, *T. mume*.). The substance was purified and crystallized by our method. This crystal was colorless and short-pillar shaped. The melting point of this substance is 177-178℃ (dec.).

The substance was soluble in ethanol, methanol, acetone and water; slightly soluble in ether, butanol, propanol and ethyl acetate; insoluble in ligroine, petroleum ether, chloroform and xylene.

The aqueous solution of this substance was acidic. And this crystal dissolves in dilute sodium bicarbonate solution with bubbling.

In the paper chromatography, the *Rf* value of the substance was 0.17 at *iso*propanol-ammonia-water (8 : 1 : 1) and 0.74 at butanol-acetic acid-water (4 : 1 : 1). The spot of this substance on the paper chromatogram was detected by 0.05 per cent ninhydrin, 1 per cent $FeCl_3$, ammoniacal silver nitrate solution, 0.05 per cent BPB.

The aqueous solution of the substance exhibited no absorption maximum between the wave length of 230 mμ and 310 mμ.

This substance promoted the elongation of the pea and flax root, and stimulated the development of axillary buds of the bean in the concentration of 10-20 p.p.m.. And the development of aerial root of the tomato and thickening of *Bryophyllum* mesophyll were caused by the treatment with the lanolin paste which contains the substance.

From these natures, the purified active substance was quitely different from IAA. This substance will be named "Taphrinin" by the authors.

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